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**Salmonella Real Time PCR Kit User Manual**
**For In Vitro Diagnostic Use Only**

REF **DD-0034-01**

*For use with LightCycler1.0/2.0 Instrument*


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**1. Intended Use**

Salmonella real time PCR kit is used for the detection of Salmonella in stool or water samples by using real time PCR systems.

**2. Principle of Real-Time PCR**

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

**3. Product Description**

Salmonella is a genus of bacteria that are a major cause of foodborne illness throughout the world. The bacteria are generally transmitted to humans through consumption of contaminated food of animal origin, mainly meat, poultry, eggs and milk.

The symptoms of Salmonella infection usually appear 12–72 hours after infection, and include fever, abdominal pain, diarrhoea, nausea and sometimes vomiting. The illness usually lasts 4–7 days, and most people recover without treatment. However, in the very young and the elderly, and in cases when the bacteria enter the bloodstream, antibiotherapy may be needed.

Salmonella real time PCR Kit contains a specific ready-to-use system for the detection of the Salmonella by polymerase chain reaction (PCR) in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of the Salmonella DNA including S.enterica, S.bongoru, S.subterranae and suspecies of S.enterica. Fluorescence is emitted and measured by the real time systems´ optical unit during the PCR. The detection of amplified Salmonella DNA fragment is performed in fluorimeter **channel 530nm** with the fluorescent quencher BHQ1. DNA extraction buffer is available in the kit and excreta or water samples are used for DNA extraction. In addition, the kit contains a system to identify possible PCR inhibition by measuring the 560nm fluorescence of the internal control (IC).

**4. Kit Contents**

| Ref. | Type of Reagent | Presentation 25rxns |
|---|---|---|
| 1 | DNA Extraction Buffer | 2 vials, 1.5ml |
| 2 | Salmonella Reaction Mix | 1 vial, 450µl |
| 3 | PCR Enzyme Mix | 1 vial, 12µl |
| 4 | Molecular Grade Water | 1 vial, 400µl |
| 5 | Internal Control (IC) | 1 vial, 30µl |
| 6 | Salmonella Positive Control | 1 vial, 30µl |

**Analysis sensitivity: $1\times10^4$ copies/ml**

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors .If you use the DNA extraction buffer in the kit, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

**5. Storage**

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (>3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Reaction mix should be stored in the dark.

**6. Additionally Required Materials and Devices**

- Biological cabinet
- Real time PCR system
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)
- Vortex mixer
- Real time PCR reaction tubes/plates
- Cryo-container
- Pipets (0.5µl – 1000µl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and Freezer
- Tube racks

**7. ⚠Warnings and Precaution**

- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Quickly prepare the reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.

**8. Sample Collection, Storage and transportation**

- Collect samples in sterile tubes;
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

**9. Procedure**

**9.1 DNA-Extraction**

DNA extraction buffer is supplied in the kit. Please thaw the buffer thoroughly and spin down briefly in the centrifuge before use. It's better to use commercial kits for nucleic acid extraction.

**9.1.1 Stool samples**

1) Take about 50mg samples to a 1.5ml tube; add 1.0ml normal saline then vortex vigorously. Centrifuge the tube at 13000rpm for 2 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet.
2) Add 100µl DNA extraction buffer, close the tube then resuspend the pellet with vortex vigorously. Spin down briefly in a table centrifuge.
3) Incubate the tube for 10 minutes at 100°C.
4) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

**9.1.2 Water samples**

1) Take 3ml water to a tube, Centrifuge the tube at 13000rpm for 2 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet.
2) Add 100µl DNA extraction buffer, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.
3) Incubate the tube for 10 minutes at 100°C.
4) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

**Attention:**

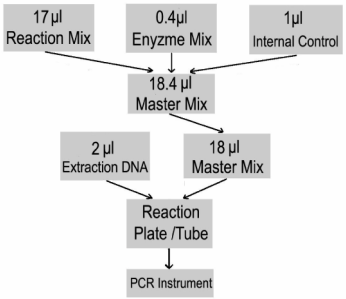
**A.** During the incubation, make sure the tube is not open，as the vapor will volatilize into the air and may cause contamination if the sample is positive.
**B.** The extraction sample should be used in 3 hours or store at -20°C for one month.
**C.** Different DNA extraction kits are available. You may use your own extraction systems or the commercial kit based on the yield. For the DNA extraction, please comply with the manufacturer's instructions.

**9.2 Internal Control**

It is necessary to add internal control (IC) in the reaction mix. Internal Control (IC) allows the user to determine and control the possibility of PCR inhibition.
Add the internal control (IC) 1µl/rxn and the result will be shown in the 560nm.

**9.3 PCR Protocol**

The Master Mix volume for each reaction should be pipetted as follows:




※PCR system without 560nm channel may be treated with 1µl Molecular Grade Water instead of 1µl IC.

1) The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of the controls，standards and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix the master mix completely then spin down briefly in a centrifuge.
2) Pipet **18µl** Master Mix with micropipets of sterile filter tips to each *Real time* PCR reaction plate/tube. Then separately add **2µl** DNA sample, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:

| | |
|---|---|
| 37°C for 2min | 1cycle |
| 94°C for 2min | 1cycle |
| 93°C for 5sec, 60°C for 30sec ( Fluorescence measured at 60°C) | 40cycles |

| Selection of fluorescence channels | |
|---|---|
| 530nm | Target Nucleic Acid |
| 560nm | IC |

**10.Threshold setting:** Choose **Arithmetic** as back ground and **none** as Noise Band method, then adjust the Noise band just above the maximum level of molecular grade water, and adjust the threshold just under the minimum of the positive control.

**11.Quality control:** Negative control, positive control and internal control must be performed correctly, otherwise the sample results is invalid.

| Channel / Control | Crossing point value | |
|---|---|---|
| | 530nm | 560nm |
| Molecular Grade Water | Blank | 25~35 |
| Positive Control(qualitative assay) | ≤35 | —— |

**12. Data Analysis and Interpretation**

The following results are possible:

| | Crossing point value | | Result Analysis |
|---|---|---|---|
| | 530nm | 560nm | |
| 1# | Blank | 25~35 | Below the detection limit or negative |
| 2# | ⩽35 | —— | Positive |
| 3# | 35～40 | 25~35 | Re-test; If it is still 35~40, report as 1# |
| 4# | Blank | Blank | PCR Inhibition; No diagnosis can be concluded. |

For further questions or problems,please contact our technical support at **trade@liferiver.com.cn**